Straightforward Synthesis of Labeled and Unlabeled Pyrimidine d4Ns via 2′,3′-Diyne *seco* Analogues through Olefin Metathesis Reactions

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The synthesis of dideoxynucleosides (ddNs) or didehydro-dideoxynucleosides (d4Ns) from nucleosides has been extensively reviewed. While previously described methods are based on the modification of the 2′- and/or 3′-OH group of the intact ribose moiety, the use of a ring-closing metathesis (RCM) for the formation of the unsaturated cyclic system of nucleosides could be a straightforward approach to the d4Ns. Thus, as part of our drug labeling program, this paper reports a straightforward synthesis of 2′,3′-didehydro-2′,3′-dideoxyuridine (d4U) and [1′,2′,3′,4′,5′-$^{13}C_5$,6-^{13}C,1,3-$^{15}N_2$]d4T using the RCM protocol. This paper discusses the preparation of nucleoside dienes and the activity of ruthenium-based metathesis catalysts.

Introduction

In recent years, a number of dideoxynucleosides, such as ddC (2′,3′-dideoxycytidine, **1**), ddI (2′,3′-dideoxyinosine, **2**), d4T (2′,3′-didehydro-3′-deoxythymidine, **3**), 3TC (β-L-3′-deoxy-3′-thicytidine, **4**), and AZT (3′-azido-3′-deoxythymidine, **5**), have been approved by the FDA as potent and selective anti-HIV agents[1] (Figure 1).

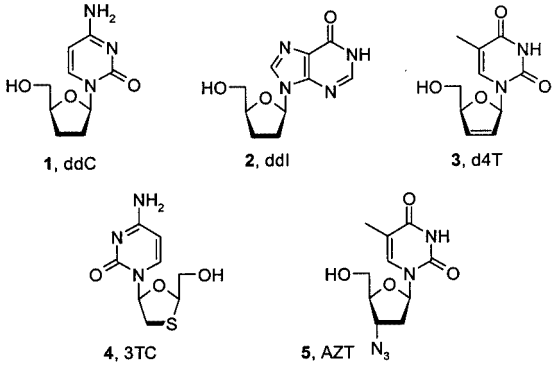

Figure 1. Structures of ddC (**1**), ddI (**2**), d4T (**3**), 3TC (**4**), and AZT (**5**)

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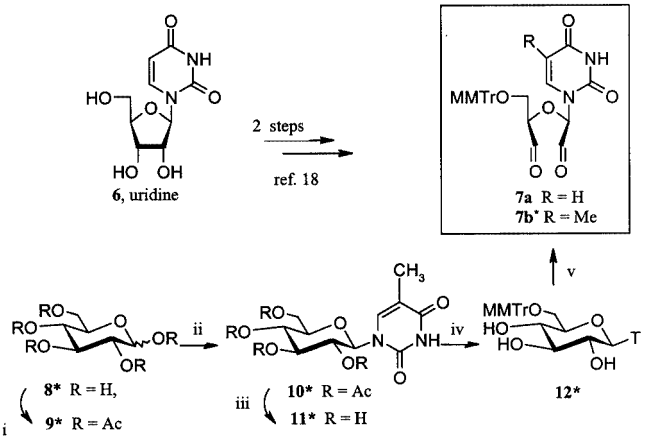
This finding has triggered new developments in the chemistry of these and related compounds and analogues.[2] For instance, labeled nucleosides, recently reviewed by Lagoja et al.[3a] and Milecki et al.,[3b] are of great interest for pharmacokinetics studies by NMR spectroscopy. In fact, NMR analysis has not only yielded atomic resolution structures of macromolecules in solution but has also enabled the study of living systems, giving rise to whole new fields including in vivo NMR spectroscopy[4] and magnetic resonance imaging (MRI). The power of in vivo NMR spectroscopy lies not in determining structures de novo, but in observing changes in the structures of biological macromolecules and in their interaction with other cellular components. These techniques rely extensively on the introduction of isotopically labeled molecules. A new high-yielding ring contraction has recently been discovered and is the key intermediate for a straightforward synthesis of [1′,2′,3′,4′,5′-$^{13}C_5$,6-^{13}C,1,3-$^{15}N_2$]d4T. Nevertheless, improvements in the efficiency of the synthesis of those d4Ns are still a particularly important issue due to the need to limit costs but also to develop several corresponding ^{13}C- and/or ^{15}N-containing analogues.[5]

The synthesis of dideoxynucleosides (ddNs) or didehydro-dideoxynucleosides (d4Ns) from nucleosides has been extensively reviewed.[6] It has been mainly achieved through a radical reaction (Barton deoxygenation),[7] a fragmentation of 2′,3′-cyclic orthoformates (Eastwood olefination)[8] or 2′,3′-cyclic thionocarbonates (Corey−Winter reaction),[9] a reductive elimination of 2′(3′)-acetoxy-3′(2′)-halo derivatives (Mattocks reaction),[10] a deoxygenation of 5′-*O*-protected nucleoside 2′,3′-dimesylate by treatment with Te^{2-} or $ArSe^-$,[11] stereoselective coupling from 2-phenyl-

seleno[12] or 2′-phenylsulfeno sugars.[13] While those methods are based on the modification of the 2′- and/or 3′-OH group of the intact ribose moiety, the use of a ring-closing metathesis (RCM) − a promising tool in nucleoside chemistry[14−16] − for the formation of the unsaturated cyclic system of nucleosides could be a straightforward approach to d4Ns. Incidentally, it is noteworthy that during the course of our investigation, Ewing et al.[16] reported a similar approach to unlabelled d4T. Thus, as part of our drug labeling program, this contribution reports a straightforward synthesis of 2′,3′-didehydro-2′,3′-dideoxyuridine (d4U) and [1′,2′,3′,4′,5′-$^{13}C_5$,6-^{13}C,1,3-$^{15}N_2$]d4T. This paper exemplifies also the activity of ruthenium-based metathesis catalysts.[17]

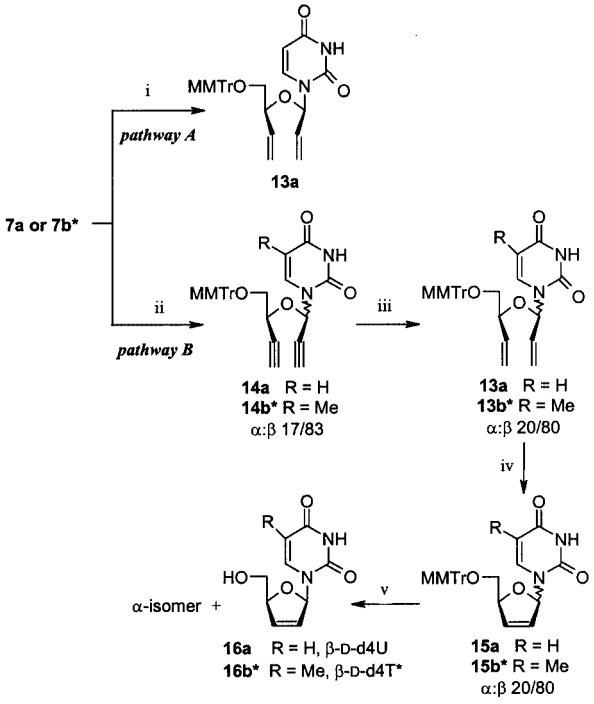
Results and Discussion

Uridine (**6**) was converted into the dialdehyde **7a** by a reported method (Scheme 1).[18] For the labeled counterpart **7b**, after acetylation of [$^{13}C_6$]-D-glucose (**8**) to the corresponding [$^{13}C_6$]pentaacetyl-D-glucose (**9**), a Vorbrüggen condensation with [6-^{13}C,1,3-$^{15}N_2$]thymine was carried out to obtain [1′,2′,3′,4′,5′,6′-$^{13}C_6$]-(2′,3′,4′,6′-tetraacetyl-β-D-glucopyranosyl)-[6-^{13}C,1,3-$^{15}N_2$]thymine (**10**). After deprotection to the corresponding β-D-glucopyranoside (**11**), monomethoxytritylation yielded the 1′,6′-substituted β-D-glucopyranoside (**12**). Oxidative ring opening by cleavage of the α-glycol group with periodic acid or with lead tetraacetate has been described by Malaprade[19a] and Criegee,[19b] respectively. Thus, to obtain the nucleoside dialdehyde (**7b**)[20] from the corresponding 6′-protected glucopyranoside (**12**), an oxidation with 3 equiv. of lead tetraacetate was carried out.



Scheme 1. Compounds indicated with * are fully ^{13}C-labeled at the sugar moiety and at the 6-^{13}C and 1,3-$^{15}N_2$ positions on the base; reagents: (i) Ac_2O, pyridine 98%; (ii) [6-^{13}C,1,3-$^{15}N_2$]thymine, BSA, TMSOTf, $ClCH_2CH_2Cl$, 98%; (iii) NH_3/MeOH, 98%; (iv) MMTrCl, pyridine, Et_3N, 98%; (v) $Pb(OAc)_4$, CH_2Cl_2, 92%

Having in hand the dialdehydes (**7a** and **7b**), our first aim (pathway **A**) was to synthesize the diene **13a** directly from the corresponding dialdehyde **7a** (Scheme 2).



Scheme 2. Compounds indicated with * are fully ^{13}C-labeled at the sugar moiety and at the 6-^{13}C and 1,3-$^{15}N_2$ positions on the base; reagents: (i) $Ph_3P^+CH_3Br^-$, 12-crown-4, *n*BuLi, THF, −78 °C to room temp. 2 d; 18%; (ii) 3 equiv. $CH_3COC(N_2)P(O)(OMe)_2$, 4 equiv. K_2CO_3, MeOH, 0 °C to room temp., **14a**: 79%, **14b***: 70%; (iii) H_2, Lindlar Pd, quinoline, room temp. 18 h, **13a**: 78%, **13b***: 82%; (iv) Nolan's catalyst (**17**, 10 mol %), benzene, reflux, 4.5 h, **15a**: 89%, 15b*: 90%; (v) Dowex® 50 W, H^+ form in MeOH, room temp. 1 h, **16a**: 86%, **16b***: 85%

Several attempts (Table 1) were carried out in order to obtain the diolefins **13** under optimized conditions.

The overall yield remained very low despite employing several strategies, such as the classical Wittig olefination conditions ($Ph_3P^+CH_3Br^-$, *n*BuLi, THF, −78 °C to room temp.) (Entry 1), an alkene synthesis using CH_2Br_2/Zn in the presence of a Lewis acid (Entry 2),[21] the Peterson reaction (Entry 3),[22] or the use of the Tebbe reagent (Entry 4).[23] By performing the olefination step with $Ph_3P^+CH_3Br^-$ and *t*BuOK in toluene (Entry 5), we obtained a mixture of inseparable isomers in a 6:4 ratio, even at low temperature. We presume that as the proton 1′-H is very labile, the isomerisation at the anomeric position occurs in alkaline media. The ratio of α- and β-isomers was readily determined by ^{1}H NMR spectroscopy. The best results were obtained by performing a Wittig olefination ($Ph_3P^+CH_3Br^-$, *n*BuLi, THF, −78 °C to room temp.) in the presence of a catalytic amount of 12-crown-4 (Entry 6).[24] Under these conditions, the desired diene **8a** was isolated as a single isomer (β-isomer) but in only 18% yield. Nevertheless, this optimized Wittig olefination with triphenylphosphonium bromide is not efficient enough for labeling purposes.

Table 1. Preparation of olefin **13a** and diyne **14a** from dialdehyde **7a**

Entry	Substrate	Product (yield)	Conditions
1	**7a**	**13a** (< 5%)	$Ph_3P^+CH_3Br^-$, *n*BuLi, THF, −78 °C to room temp.
2	**7a**	**13a** (< 5%)	CH_2Br_2, Zn (with or without $TiCl_4$)
3	**7a**	**13a** (< 5%)	1) $TMSCH_2MgCl$; $BF_3{\cdot}Et_2O$
4	**7a**	**13a** (< 5%)	$Cp_2Ti(\mu\text{-}Cl)(\mu\text{-}CH_2)AlMe_2$, THF
5	**7a**	**13a** (10%) α/β = 4:6[a]	$Ph_3P^+CH_3Br^-$, *t*BuOK, toluene, −78 °C to room temp.
6	**7a**	**13a** (18%)	$Ph_3P^+CH_3Br^-$, *n*BuLi, 12-crown-4, THF, −78 °C to room temp.
7	**7a**	**14a** (79%) α/β = 20:80[a]	3 equiv. $CH_3COC(N_2)P(O)(OMe)_2$, 4 equiv. K_2CO_3, MeOH, 0 °C to room temp.

[a] Mixture of isomers separable in the last step.

In an attempt to increase the yield of the diene, we envisaged an alternative synthesis using the partial reduction of the corresponding diyne (pathway **B**). Based on work first described by Ohira,[25] the dialdehyde **7a** or **7b** was transformed into the corresponding diyne (**14a** or **14b**) upon reaction with the in situ generated anion of dimethyl diazomethylphosphonate, which was prepared by acyl cleavage of dimethyl (1-diazo-2-oxopropyl)phosphonate [$CH_3COC(N_2)P(O)(OMe)_2$] under argon.[26] This mild method has been applied previously, without any reported racemization, for the functionalization of chiral α-alkoxy aldehydes[27] or chiral α-amino aldehydes.[28] To the best of our knowledge, this is the first time that this method has been used to generate a diyne from a dialdehyde compound, and applied to nucleoside chemistry. Thus, the dialdehyde (**7a** or **7b**) was treated with 3 equiv. of dimethyl (1-diazo-2-oxopropyl)phosphonate in methanol in the presence of 4 equiv. of K_2CO_3 at 0−23 °C for 5 h (Entry 7, Table 1). The diacetylene derivative (**14a** or **14b**) was isolated in good yield but as a mixture of α- and β-isomers in a 17:83 ratio. Despite various optimization experiments where solvent (1-propanol, butanol), temperature and quantities or nature of the base (K_2CO_3, Cs_2CO_3) were modified, racemization was always observed. Although the mixture of β- and α-isomers could not be separated by classical chromatography techniques at this stage, the desired β-d4Ns (**16a** and **16b**) could be isolated, without any problem, during the last step of this synthesis. A catalytic hydrogenation of (**14a** or **14b**) using Lindlar catalyst in the presence of quinoline afforded the desired diene **13a** or **13b**.

With diene **13**, as the unique isomer (or as an α/β mixture) at hand, we turned our attention to establishing the best conditions for ring-closing metathesis. In this area, ring-closing metathesis has mainly been applied to the synthesis of oxacycloalkenes.[29] Based on our previous results illustrating the user-friendly character of new ruthenium−carbene species bearing one imidazol-2-ylidene ligand (**17**, Figure 2) and on its known tolerance towards an array of polar groups (particularly the amide group), we were prompted to probe the performance of **17** in this particular application. RCM of the bis(olefinic) compound **13** to the corresponding oxacycloalkene **15** was carried out successfully in benzene at 80 °C with 10 mol % of **17** in 89% yield.[30]

Finally, deprotection of the unlabeled 2′,3′-didehydro-2′,3′-dideoxy-5′-*O*-monomethoxytrityluridine (**15a**), by acidic hydrolysis (Dowex® 50 W, H^+ form in MeOH, 86%) afforded, after a chromatographic purification, the β-D-d4U (**16a**) as a unique stereoisomer. This approach (pathway **B**) has been successfully applied to the synthesis of labeled [1′,2′,3′,4′,5′-$^{13}C_5$,6-^{13}C,1,3-$^{15}N_2$]d4T (**16b**, Figure 3). The final compounds **16a**,**b** have similar optical and physical data to the known unlabeled species.

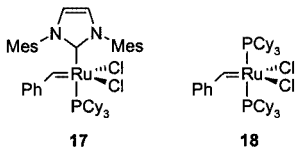


Figure 2. Ruthenium catalysts for RCM

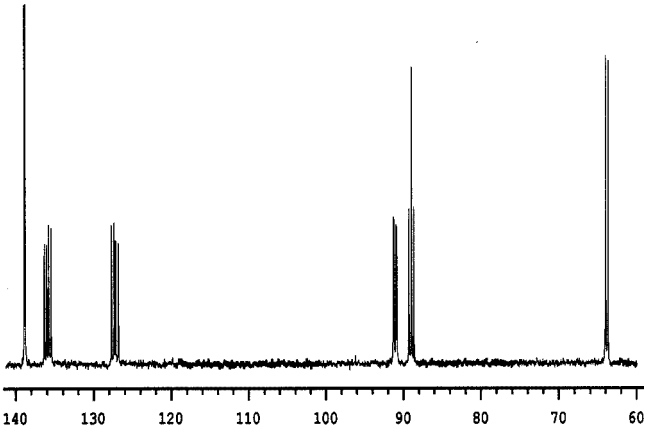


Figure 3. ^{13}C NMR spectrum of labeled d4T (125 MHz, $[D_4]$MeOH)

Conclusion

In summary, we have described the preparation of enantiomerically pure unsaturated nucleosides such as 2′,3′-didehydro-2′,3′-dideoxyuridine (d4U) and labeled [1′,2′,3′,4′,5′-$^{13}C_5$,6-^{13}C,1,3-$^{15}N_2$]d4T, using an RCM strategy. We have realized the direct preparation of a diyne from a dialdehyde using a mild and very useful method that certainly warrants further study. The ring-closing metathesis, which was performed with **17**, represents an excellent tool for RCM by combining the activity of the classical molybdenum systems with the stability and tolerance of ruth-

enium-based catalysts. This straightforward procedure offers an alternative and valuable method that can be applied to the synthesis of related labeled pyrimidine and purine nucleosides and analogues. This area of investigation is currently ongoing in our laboratories.

Experimental Section

Materials and General Methods: THF and benzene were distilled from sodium/benzophenone ketyl immediately prior to use. Dichloromethane and dichloroethane were distilled from CaH_2 and methanol from magnesium turnings. The starting products are commercially available. Isotopically labeled materials were obtained from Campro Scientific. [6-^{13}C,1,3-$^{15}N_2$]thymine[31], [1′,2′,3′,4′,5′,6′-$^{13}C_6$]-(2′,3′,4′,6′-tetraacetyl-β-D-glucopyranose (**9**)[32] and (monomethoxytrityl)uridinedialdehyde (**7a**)[33] were prepared according to literature procedures. The reactions were monitored by thin-layer chromatography (TLC) analysis on silica gel plates (Kieselgel 60 F_{254}, E. Merck). Compounds were visualized by UV irradiation and/or spraying with a solution of phosphomolybdic acid, followed by charring at 150 °C. Column chromatography was performed on Silica Gel 60 M (0.040−0.063 mm, E. Merck). Melting points were determined with a Büchi SMP-20 capillary melting point apparatus. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker AVANCE DPX 250 Fourier Transform spectrometer at 250 MHz (^{13}C, 62.9 MHz), or with a Varian Unity 500 spectrometer (^{13}C, 125.527 MHz, ^{15}N, 50.586 MHz). ^{13}C data are referenced to TMS and ^{15}N data to liquid NH_3. Mass spectra were recorded with Perkin−Elmer SCIEX API-300 (heated nebulizer) spectrometer. HRMS spectra were recorded at the Rega Institute, Katholieke Universiteit Leuven, using a quadrupole time-of-flight mass spectrometer (Q-Tof-2, Micromass, Manchester, UK) equipped with a standard electrospray ionization (ESI) interface. Samples were infused in a 2-propanol/water (1:1) mixture at 3 μL/min. HPLC to purify d4T was performed on an HS Prep100 BDS C18 8u (250 × 10 mm) column, using H_2O/MeOH (93:7) as eluent. The nomenclature of the obtained compounds is in accordance with the IUPAC rules and was checked with Autonome.[34] The numbering and assignment of the chemical shifts for compounds **13** and **14** are related to the corresponding ribose derivatives. PE = petroleum ether.

[1′,2′,3′,4′,5′,6′-$^{13}C_6$]-(2′,3′,4′,6′-Tetraacetyl-β-D-glucopyranosyl)-[6-^{13}C,1,3-$^{15}N_2$]-thymine (10): BSA (2.4 mL, 10 mmol) was added to a suspension of [6-^{13}C,1,3-$^{15}N_2$]thymine (0.50 g, 4.27 mmol) and [$^{13}C_6$]glucosepenta-*O*-acetate (**9**; 1.63 g, 4.27 mmol) in dichloroethane (40 mL). The mixture was stirred under nitrogen at ambient temperature for 20 min to give a clear and colorless solution. After addition of TMSOTf (3.60 mL, 20 mmol) under nitrogen the reaction mixture was heated under reflux for 4 h. The resultant brown mixture was cooled to ambient temperature and the solvents were evaporated in vacuo to give an oil, which was diluted in ethyl acetate (200 mL) and washed with $NaHCO_3$ (sat.) (150 mL) and brine (2 × 100 mL). After drying with Na_2SO_4 and evaporating the solvent, the resultant oil was purified by column chromatography (silica gel, EtOAc/PE, 2:1). Yield: 1.95 g (98%). R_f (EtOAc/PE, 3:2) = 0.70. M.p. 156−158 °C (MeOH). ^{13}C NMR (125 MHz, $CDCl_3$): δ = 12.2 (s, CH_3), 20.2, 20.3, 20.4, 20.6 (4 × CH_3 Ac), 61.5 (d, J = 44 Hz, 6′-CH_2), 67.8 (t, J = 44 Hz, 4′-CH), 69.2 (t, J = 44 Hz, 2′-CH), 72.7 (t, J = 44 Hz, 3′-CH), 75.0 (t, J = 44 Hz, 5′-CH), 80.3 (dd, J = 15, J = 46 Hz, 1′-CH), 112.1 (d × d, J = 6, J = 64 Hz, 5-CH), 134.5 (d, J = 12 Hz, 6-CH), 150.6 (t, J = 12 Hz, 2-C), 163.5 (d, J = 12 Hz, 4-C), 169.5, 169.6, 169.7, 170.5 (4 × C=O) ppm. Exact mass: $C_{12}{}^*C_7H_{24}{}^*N_2O_{11}Na$: calcd. 488.1452; found 488.1460.

[1′,2′,3′,4′,5′,6′-$^{13}C_6$]-(β-D-Glucopyranosyl)-[6-^{13}C,1,3-$^{15}N_2$]thymine (11): A solution of the tetra-*O*-acetyl protected nucleoside (**10**; 4.2 mmol) in MeOH (10 mL) saturated with NH_3 was stirred overnight at ambient temperature. After removal of the solvent in vacuo the precipitate was recrystallized from dichloromethane. Yield: 1.17 (98%). R_f (CH_2Cl_2/MeOH, 2:1) = 0.76. M.p. 270−271 °C (MeOH). ^{13}C NMR (125 MHz, [D_6]DMSO): δ = 12.1 (s, CH_3), 60.9 (d, J = 43 Hz, 6′-CH), 69.6 (t, J = 43 Hz, 4′-CH), 70.8 (t, J = 43 Hz, 2′-CH), 77.0 (t, J = 43 Hz, 3′-CH), 80.0 (t, J = 43 Hz, 5′-CH), 82.4 (dd, J = 14, J = 45 Hz, 1′-CH), 109.7 (dd, J = 6, J = 64 Hz, 5-CH), 137.0 (d, J = 12 Hz, 6-CH), 151.3 (t, J = 12 Hz, 2-C), 164.1 (d, J = 11 Hz, 4-C) ppm. ^{15}N NMR (50 MHz, [D_6]DMSO): δ = 143.0 (N-1), 157.9 (N-3) ppm. Exact mass: $C_3{}^*C_7H_{17}{}^*N_2O_7$: calcd. 284.1055; found 284.1061.

[1′,2′,3′,4′,5′,6′-$^{13}C_6$]-6′-*O*-Monomethoxytrityl-(β-D-glucopyranosyl)-[6-^{13}C,1,3-$^{15}N_2$]thymine (12): A mixture of glucopyranosyl-nucleoside (**11**; 1.0 g, 3.5 mmol), and MMTrCl (2.16 g, 7.0 mmol) in pyridine (20 mL) and triethylamine (5 mL) was stirred under an inert gas at ambient temperature for 48 h. After removal of the solvent in vacuo, the resultant oil was coevaporated with toluene (4 × 2 mL). The resultant foam was purified by column chromatography (silica gel, CH_2Cl_2/MeOH, 9:1). Yield: 1.95 g (98%); R_f (CH_2Cl_2/MeOH, 9:1) = 0.56. M.p. 170−172 °C (CH_2Cl_2). ^{13}C NMR (125 MHz, [D_6]DMSO): δ = 12.6 (s, CH_3), 55.1 (s, CH_3O), 64.0 (d, J = 45 Hz, 6′-CH), 69.8 (t, J = 45 Hz, 4′-CH), 70.7 (t, J = 45 Hz, 2′-CH), 76.9 (t, J = 45 Hz, 3′-CH), 78.0 (t, J = 45 Hz, 5′-CH), 82.4 (d × d, J^1 = 14, J^2 = 45 Hz, 1′-CH), 85.8 (C, MMTr), 109.6 (d × d, J^1 = 12, J^2 = 64 Hz, 5-CH), 113.3 (s, 2 CH, AA′BB′, MMTr), 126.9−130.3 (CAr, MMTr), 135.4 (s, 1 C, AA′BB′, MMTr), 136.8 (d, J = 12 Hz, 6-CH), 144.7 (s, 2 × 1 C, MMTr), 151.3 (t, J = 12 Hz, 2-C), 158.3 (s, 4 C, AA′BB′, MMTr), 163.3 (d, J = 11 Hz, 4-C) ppm. Exact mass: $C_{24}{}^*C_7H_{32}{}^*N_2O_8Na$: calcd. 592.2232; found 592.2297.

[1′,2′,3′,4′,5′-$^{13}C_5$]-5′-*O*-Monomethoxytrityl-[6-^{13}C,1,3-$^{15}N_2$]thymidinedicarbaldehyde (7b): $Pb(OAc)_4$ (4.21 g, 9.5 mmol) was added under nitrogen and with ice cooling to a solution of the 1′,6′-disubstituted β-D-glucopyranoside (**12**; 1.8 g, 3.17 mmol) in dry CH_2Cl_2 (30 mL) and Et_3N (1 mL). The solution became warm and turned yellow. After 2 h, a colorless precipitate of $Pb(OAc)_2$ was formed. Stirring was continued at room temperature for another 5 h. After reducing the volume of the solvent in a rotary evaporator, the residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH, 95:5). MS measurements confirmed the structure of the resultant foam. Because of the complicated equilibrium observed by NMR spectroscopy it was not possible to interpret the NMR spectra in a straightforward way. Yield: 1.52 g (92%). R_f (CH_2Cl_2/MeOH, 95:5) = 0.74. Exact mass: $C_{23}{}^*C_6H_{26}{}^*N_2O_8Na$: calcd. 545.1774; found 545.1766.

1-[1-({1-[(Monomethoxytrityl)methyl]-2-propenyl}oxy)-2-propenyl]-uracil (13a) by Wittig Reaction: *n*BuLi (0.94 mL, 1.6 M in *n*-hexane) was slowly added to a stirring solution of $Ph_3P^+CH_3Br^-$ (5.2 g, 14.6 mmol) and 12-crown-4 (0.51 g, 2.89 mmol) in THF (50 mL) under argon at −78 °C. The mixture was allowed to reach 0 °C, then cooled again to −78 °C and a solution of the crude dialdehyde **7a** (2.92 mmol) in THF (5 mL) was added. The reaction mixture was stirred for 48 h at room temperature, then water was added and the mixture extracted with CH_2Cl_2 (3 ×). Drying ($MgSO_4$) of the organic phase and removal of the solvent under vacuum gave

the crude product, which was then purified by column chromatography (silica gel, toluene/ethyl acetate, 7:3 with 1% of Et_3N), to give the diene **13a.** Yield: 268 mg (18%), only β-isomer. R_f (PE/EtOAc, 1:1) = 0.3. ^1H NMR (250 MHz, $CDCl_3$): δ = 3.09 (dd, *J* = 3.1, *J* = 10.3 Hz, 1 H, 5′-H), 3.34 (dd, 1 H, *J* = 8.17, *J* = 10.3 Hz), 3.78 (s, 3 H, CH_3), 3.90 (m, 1 H, 4′-H), 5.27−5.35 (m, 3 H, 6′-H_2, 7′-H_1), 5.53−5.67 (m, 2 H, 3′-H, 7′-H_1), 5.73 (s, 1 H, 5-H), 5.81 (ddd, 1 H, *J* = 3.5, *J* = 10.4, *J* = 17.2 Hz, 2′-H), 6.26 (m, 1 H, 1′-H), 6.83 (m, 2 H, Ar), 7.15−7.47 (m, 12 H, Ar), 7.49 (d, *J* = 8.15 Hz, 1 H, 6-H) ppm. ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 55.3 (CH_3O), 66.1 (5′-CH_2), 78.5 (4′-CH), 80.4 (1′-CH), 86.7 (C^{IV}, MMTr), 103.2 (5-CH), 113.1 (CH, ar), 119.3 (CH_2=), 120.9 (CH_2=), 127.1, 127.9, 128.4, 128.5, 130.4 (CH, Ar), 132.9 (2′-CH), 133.6 (3′-CH),135.4 (C^{IV}), 140.9 (6-CH), 144.4, 151.2 (C^{IV}), 158.7, 163.7 (C=O) ppm. MS IS: *m*/*z* = 533 [M + Na]. Exact mass: $C_{31}H_{30}N_2O_5Na$: calcd. 533.2052; found 533.2059. IR: $\tilde{\nu}_{max}$ = 2925, 1686, 1508, 1250, 1072 cm^{-1}.

1-[1-({1-[(Monomethoxytritylmethoxy)methyl]-2-propynyl}oxy)-2-propynyl]uracil (14a) and [1′,2′,3′,4′,5′-$^{13}C_5$]-1-[1-({1-[(Monomethoxytritylmethoxy)methyl]-2-propynyl}oxy)-2-propynyl]-[6-^{13}C,1,3-$^{15}N_2$]thymine (14b). General Procedure: Anhydrous K_2CO_3 (244.5 mg, 1.77 mmol) was added to a solution of dry dialdehyde **7b** (0.443 mmol) in dry MeOH (10 mL) at 0 °C under argon. After stirring for 10 min, dimethyl (1-diazo-2-oxopropyl)phosphonate (255 mg, 1.33 mmol) was added. The mixture was allowed to reach room temperature and was stirred until TLC showed complete conversion of the substrate (4 h). The reaction mixture was then diluted with ethyl acetate (25 mL) and washed with an aqueous solution of $NaHCO_3$ (5%). The aqueous layer was washed with ethyl acetate (3 ×), and the combined organic layers were dried with anhydrous $MgSO_4$, filtered and concentrated in vacuo. Purification by flash chromatography (silica gel, PE/EtOAc, 3:2) gave the dialkyne **14** in 79% yield as an inseparable mixture of β/α stereoisomers (80:20).

14a: R_f (PE/EtOAc, 1:1) = 0.31. ^1H NMR (250 MHz, $CDCl_3$) (β-isomer): δ = 2.53 (d, *J* = 2 Hz, 1 H, ≡CH), 2.76 (d, *J* = 2 Hz, 1 H, ≡CH), 3.28 (dd, *J* = 3.8, *J* = 10.4 Hz, 1 H, 5′-H), 3.46 (dd, *J* = 7.8, *J* = 10.4 Hz, 1 H), 3.77 (s, 6 H, CH_3), 4.17 (m, 1 H, 4′-H), 5.82 (d, *J* = 8.1 Hz, 1 H, 5-H), 6.75 (d, *J* = 2 Hz, 1 H, 1′-H), 6.79−6.85 (m, 3 H, Ar), 7.19−7.48 (m, 10 H, Ar), 7.64 (d, *J* = 8.2 Hz, 1 H, 6-H) ppm. ^{13}C NMR (62.9 MHz, $CDCl_3$) (β-isomer): δ = 55.3 (CH_3O), 65.7 (5′-CH_2), 67.8 (4′-CH), 72.5 (1′-CH), 76.6 (≡CH), 78.8 (C^{IV}), 86.9 (C^{IV}, MMTr), 104.1 (5-CH), 111.0 (≡CH), 113.3 (CH, ar), 127.1, 128.0, 128.4, 128.5, 130.4 (CH, Ar), 140.2 (6-CH), 144.2, 150.3 (C^{IV}), 158.8, 163.1 (C=O) ppm. MS IS: *m*/*z* = 529 [M + Na]. Exact mass: $C_{31}H_{26}N_2O_5Na$: calcd. 529.1739; found 529.1735. IR: $\tilde{\nu}_{max}$ = 3190, 3058, 2944, 2836, 2129, 1690, 1509, 1250 cm^{-1}.

14b: Yield: 163 mg (70%), β/α stereoisomer (83:17). R_f (PE/EtOAc, 1:1) = 0.33. ^{13}C NMR (125 MHz, $CDCl_3$) (β-isomer): δ = 65.6 (d, $J_{5'4'}$ = 44.9 Hz, 5′-CH_2), 67.5 (d, d, d, $J_{4'3'}$ = 75.2, $J_{4'5'}$ = 44.9, $J_{4'2'}$ = 4.9 Hz, 4′-CH), 72.0 (dd, $J_{1'2'}$ = 90.8, $J_{1'N}$ = 12.7 Hz, 1′-CH), 76.8 (dd, $J_{2'1'}$ = 88.9, $J_{1'N}$ = 12.7 Hz, 2′-C), 78.0 (d, $J_{3'4'}$ = 75.2 Hz, 3′-C), 135.5 (d, J_{6N} = 12.7 Hz, 6-CH) ppm. Exact mass: $C_{26}{}^*C_6H_{28}{}^*N_2O_5Na$: calcd. 551.2038; found 551.2109.

1-[1-({1-[(Monomethoxytrityl)methyl]-2-propenyl}oxy)-2-propenyl]-uracil (13a) or [1′,2′,3′,4′,5′-$^{13}C_5$]-1-[1-({1-[(monomethoxytrityl)-methyl]-2-propenyl}oxy)-2-propenyl]-[6-^{13}C,1,3-$^{15}N_2$]thymine (13b) by Hydrogenation. General Procedure: Quinoline (2 mL) and Lindlar Pd (10% w/w) were added to a solution of **14a** or **14b** (0.453 mmol) in MeOH (10 mL). The resultant suspension was hydrogenated (1 atm, room temp.) for 18 h. The reaction mixture was filtered, and the solvents were evaporated from the filtrate. The crude oil was purified by flash chromatography (silica gel, PE/EtOAc, 3:2 then 2:3) to give the diene **13a** (180 mg, 78%) or **13b**.

13b: Yield: 196 mg (82%), β/α stereoisomer (83:17). R_f (PE/EtOAc, 1:1) = 0.33. ^{13}C NMR (50 MHz, $CDCl_3$) (β-isomer): δ = 65.8 (d, $J_{5'4'}$ = 44.0 Hz, 5′-CH_2), 78.4 (d,d, $J_{4'3'}$ = 45.9, $J_{4'5'}$ = 44.9 Hz, 4′-CH), 80.4 (dd, $J_{1'2'}$ = 52.7, $J_{1'N}$ = 12.7 Hz, 1′-CH), 132.8 (d, $J_{3'4'}$ = 45.9 Hz, 3′-C), 133.6 (d, $J_{2'1'}$ = 54.7, $J_{1'N}$ = 12.7 Hz, 2′-C), 135.6 (d, J_{6N} = 12.7 Hz, 6-CH) ppm. Exact mass: $C_{26}{}^*C_6H_{28}{}^*N_2O_5Na$: calcd. 551.2038; found 551.2109.

RCM General Procedure: A solution of diene **13a** or **13b** (0.258 mmol) and ruthenium catalyst **17** (23 mg, 0.027 mmol) in degassed benzene was refluxed for 4 h (until TLC showed complete conversion of the substrate). Evaporation of the solvent followed by flash chromatography (silica gel, PE/EtOAc, 1:1) provided compound **15a** or **15b**.

15a (β-isomer):[35] Yield: 111 mg (89%). Colorless syrup which solidified on standing. R_f (PE/EtOAc, 1:1) = 0.15. ^1H NMR (250 MHz, $CDCl_3$): δ = 3.45 (m, 2 H, 5′-H), 3.79 (s, 3 H, CH_3), 4.95 (br. s, 1 H, 4′-H), 5.02 (d, *J* = 8.1 Hz, 1 H, 5-H), 5.87 (m, 1 H, 3′-H), 6.35 (m, 1 H, 2′-H), 6.83 (d, *J* = 8.8 Hz, 2 H, Ar), 7.01 (m, 1 H, 1′-H), 7.22−7.45 (m, 12 H, Ar), 7.81 (d, *J* = 8.3 Hz, 1 H, 6-H), 8.44 (br. s, 1 H, NH) ppm. ^{13}C NMR (62.9 MHz, $CDCl_3$): δ = 55.3 (CH_3O), 64.4 (5′-CH_2), 86.05 (4′-CH), 87.2 (C^{IV}, MMTr), 89.7 (1′-CH), 102.3 (5-CH), 113.3 (CH, Ar), 126.4 (3′-CH), 127.3, 128.05, 128.6, 130.6 (CH, Ar), 134.5 (C^{IV}, Ar), 134.6 (2′-CH), 141.4 (6-CH), 143.5, 143.8, 150.9, 158.8, 163.6 ppm. MS IS: *m*/*z* = 505 [M + Na]. Exact mass: $C_{29}H_{26}N_2O_5Na$: calcd. 505.1739; found 505.1746. IR: $\tilde{\nu}_{max}$ = 3196, 3059, 2933, 2874, 2837, 1686, 1608, 1461, 1253 cm^{-1}. $[\alpha]_D{}^{25}$ = 37.5 (*c* = 0.12, $CHCl_3$).

15b: Yield: 117 mg (90%), β/α stereoisomer (83:17). R_f (PE/EtOAc, 1:1) = 0.17. ^{13}C NMR (125 MHz, $CDCl_3$) (β-isomer): δ = 64.6 (d, $J_{5'4'}$ = 43.9 Hz, 5′-CH_2), 85.7 (dt, $J_{4'3'}$ = 41.2, $J_{4'5'}$ = 42.0 Hz, 4′-CH), 89.6 (dd, $J_{1'2'}$ = 43.9, $J_{1'N}$ = 12.7 Hz, 1′-CH), 126.2 (dd, $J_{1'2'}$ = 43.9, $J_{2'3'}$ = 67.4 Hz, 2′-C), 134.8 (dd, $J_{3'4'}$ = 41.2, $J_{2'3'}$ = 67.4 Hz, 3′-C), 136.1 (d, J_{6N} = 12.7 Hz, 6-CH) ppm. Exact mass: $C_{24}{}^*C_6H_{28}{}^*N_2O_5Na$: calcd. 527.2038; found 527.2040.

1-[5-(Hydroxymethyl)-2,5-dihydro-2-furanyl]uracil (d4U) (16a) and [1′,2′,3′,4′,5′-$^{13}C_5$]-1-[5-(hydroxymethyl)-2,5-dihydro-2-furanyl]-[6-^{13}C,1,3-$^{15}N_2$]thymine (Labeled d4T) (16b): Activated Dowex® 50 W (H^+ form) was added to a solution of **15a** or **15b** (0.107 mmol) in methanol (2 mL). After complete conversion of the substrate (TLC control), the insoluble materiel was filtered off, washed with methanol and the solvents were evaporated from the filtrate. Purification of the crude mixture by column chromatography (silica gel, CH_2Cl_2/MeOH, 10:1) afforded the desired compounds.

16a: Yield: 19.5 mg (86%). R_f (CH_2Cl_2/MeOH, 9:1) = 0.3. Exact mass: $C_9H_{10}N_2O_4Na$: calcd. 233.0538; found 233.0534. The physicochemical data are identical with those reported in the literature.[35]

16b: Yield: 19.8 mg (80%) (HPLC), β stereoisomer. R_f (CH_2Cl_2/MeOH, 1:1) = 0.33. M.p. 164−166 °C (Et_2O) (163−165 °C benzene[36]). ^{13}C NMR (125 MHz, [D_4]MeOH) (β isomer): δ = 63.7 (d, $J_{5'4'}$ = 41.9 Hz, 5′-CH_2), 88.9 (dt, $J_{4'3'}$ = 40.0, $J_{4'5'}$ = 41.0 Hz, 4′-CH), 91.0 (dd, $J_{1'2'}$ = 43.9, $J_{1'N}$ = 12.7 Hz, 1′-CH), 127.1 (dd, $J_{1'2'}$ = 43.9, $J_{2'3'}$ = 69.3 Hz, 2′-C), 135.7 (dd, $J_{3'4'}$ = 41.2, $J_{2'3'}$ = 68.4 Hz, 3′-C), 138.8 (d, J_{6N} = 12.7 Hz, 6-CH). ^{15}N NMR (50 MHz, [D_4]MeOH): δ = 146.3 (N-1), 151.3 (N-3) ppm. Exact mass: $C_4{}^*C_6H_{12}{}^*N_2O_4Na$: calcd. 255.0851; found 255.0840.

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